Biological Activity of Biosynthetic Rainbow Trout Growth Hormone in the Eastern Oyster, *Crassostrea virginica*

KENNEDY T. PAYNTER[1]* AND THOMAS T. CHEN[2]

[1]*Department of Zoology, University of Maryland, College Park, Maryland 20742 and* [2]*Center of Marine Biotechnology, University of Maryland, 600 E. Lombard St., Baltimore, Maryland 21202*

Abstract. Juvenile oysters were exposed to seawater containing 10^{-9}, 10^{-8}, and 10^{-7} M biosynthetic rainbow trout growth hormone (rtGH); the treatment was applied for one five-hour period per week for five weeks. At the end of the five weeks, the animals treated with the two highest concentrations of hormone were significantly longer and had dry tissue weights 50% greater than did the lowest treatment group or the control group. Continuing *in vitro* experiments on isolated oyster tissue showed that the hormone treatment significantly increased oxygen consumption. Boiled hormone had no effect. In both sets of experiments (whole animals and isolated tissues), the % dry wt (dry wt/wet wt) was significantly higher in all animals and tissues that responded to rtGH. The results demonstrate that rtGH has biological activity in oyster tissues, and this activity may be directly associated with growth regulation in the whole animal. The results further show that bivalve growth is not directly limited by environmental parameters.

Introduction

The growth of many marine and estuarine pelecypods has long been thought to be determined by environmental conditions. Many studies have revealed specific correlations between bivalve growth rate and such environmental factors as water flow, salinity, temperature, oxygen, and food availability (Dame, 1975; Hall, 1984; MacDonald and Thompson, 1985a,b; Brown, 1988; Brown and Hartwick, 1988a,b). Yet endogenous control of growth by neural or hormonal systems has been investigated in only a few species. A significant body of knowledge indicates that mollusks possess complex endocrine systems (Joosse and Geraerts, 1983), and several recent findings suggest that bivalve growth may be regulated at least in part by an endocrine system.

In general, comparative endocrinological studies of mollusks have been limited to gastropods and cephalopods, and in many of those studies, endocrine control of reproduction has been a major focus. In the snail *Lymnaea stagnalis*, however, an endogenous growth-promoting hormone has been identified and well characterized. This hormone, called the molluscan insulin-like peptide (MIP), has been isolated from *Lymnaea* neuroendocrine cells and reported to affect various aspects of growth, metabolism, and shell formation in the snail (Geraerts, 1976; Dogterom *et al.*, 1979; Dogterom and Jentjens, 1980; Dogterom and Robles, 1980; Smit *et al.*, 1988).

While much is known about the physiology of the bivalves, little attention has been paid to the endocrine regulation of their metabolism and growth. In early, rather crude experiments, for example, the growth of oysters was arrested after their cerebral ganglia had been extirpated (Galtsoff, 1964), [and similar results were obtained with the gastropod, *Crepidula fornicata*, by Lubet (1971)]. More recently, Toullec *et al.* (1988) reported that extracts of cerebral ganglia prepared from *Mytilus edulis* stimulated the incorporation of leucine into the macromolecular fraction of mantle cells that had been isolated from several bivalve species. These studies suggest that bivalve growth, like that of snails, is controlled by endocrine mechanisms that are not species specific.

Conservation of sequence and function among vertebrate growth hormone polypeptides is also well established. Furthermore, bovine growth hormone (as well as insulin) has been shown to accelerate the growth of abalone larvae (Morse, 1981, 1984), and a growth hormone

like polypeptide has been identified in the abalone (Moriyama *et al.*, 1989). Therefore, we have begun to investigate the effects of biosynthetic rainbow trout growth hormone (rtGH) on the growth and metabolism of the eastern oyster, *Crassostrea virginica*. Here we report that exogenously applied rtGH stimulates the growth of juvenile oysters and increases oxygen consumption in isolated oyster tissues.

Materials and Methods

Oyster larvae (*Crassostrea virginica*) were obtained from the Virginia Institute of Marine Science. The larvae were raised according to standard hatchery techniques. All competent larvae were induced to undergo cultchless metamorphosis (not attached to substrate) by treatment with epinephrine ($10^{-6}\ M$) in the seawater. The resulting juvenile oysters were raised in downwelling chambers in the hatchery at the Chesapeake Bay Institute (CBI) of the Johns Hopkins University.

Biosynthetic rtGH was prepared as previously described (Agellon *et al.*, 1988). The complementary DNA (cDNA) of rtGH (Agellon and Chen, 1986) was cloned and introduced into *E. coli* for large scale production of the polypeptide. GH inclusion bodies were isolated from *E. coli* cells, dissolved in a $5\ M$ guanidine hydrochloride solution, and the denatured GH polypeptide was re-natured by differential dialysis against 50 mM ammonium bicarbonate buffer (pH 10.0). The lyophilized protein was resuspended in 10 mM ammonium bicarbonate buffer pH 10.0 at a concentration of 10 mg/ml and used in appropriate amounts to inoculate the experimental seawater.

Oyster growth

In September and October 1989, during the natural growing season of the oyster in the Chesapeake Bay region, an experimental hormone treatment was begun. Four groups of 25 juvenile oysters were constructed such that the initial mean heights of the groups were not different. One group (control group) was incubated in seawater inoculated with buffer; the other three groups were incubated in seawater containing $10^{-7}\ M$, $10^{-8}\ M$, or $10^{-9}\ M$ rtGH, respectively. Each group was incubated for 5 h, once per week, in Petri dishes containing 50 ml of 1 μ-filtered ambient seawater ($\approx 8‰$) at 23°C. Initially, and before each treatment thereafter, the shell height of each oyster was measured from the umbo to the ventral shell margin. The animals were maintained in an upwelling system with a continuous flow of 100 μ filtered seawater during the entire experiment. After five weeks the oysters were sacrificed, and the final height, total weight, shell weight, wet tissue weight, and dry tissue weight were determined for each individual. Whole oyster volume was calculated as the difference between total animal weight and shell weight.

Mean values of final height, total weight, shell weight, wet tissue weight, and dry tissue weight were calculated for each treatment group. Values were statistically compared using a t-test.

Oxygen consumption of isolated tissues

Respirometry was conducted according to standard manometric procedures. Oyster gills (two gill pairs per individual) were dissected and split into gill pairs. One pair from an individual was used as a control, the other was treated with boiled or native rtGH. Therefore, oxygen consumption was compared in gills that were taken from the same animal, but that were treated differently. The gill pairs were immediately placed in manometric flasks containing 2 ml filtered seawater inoculated with buffer, boiled rtGH, or native rtGH ($10^{-6}\ M$). Native and boiled insulin and bovine growth hormone (BST) were also tested. The flasks were affixed to a differential respirometer (Gilson), immersed in the water bath (25°C), and allowed to equilibrate for 30 min. After equilibration, the respirometer system was closed and initial readings on all manometers were recorded. At 15-min intervals thereafter, manometer readings were recorded. Incubations typically lasted for 2 h. Oxygen consumption was usually linear after the first 15-min reading. Any samples not showing linear decreases in oxygen concentration were discarded. After the respirometry had been completed, the tissues were removed from the flasks, weighed, dried in a 60°C oven for 72 h, and reweighed.

Because oxygen consumption rates in gill pairs from a single individual were being compared, the oxygen consumption data were analyzed by means of a paired t-test to minimize the effects of between individual variation.

Results

After three weeks of treatment (Table 1), the oysters treated with the highest concentrations of rtGH for five weeks ($10^{-7}\ M$ and $10^{-8}\ M$) were significantly larger with respect to shell height and dry tissue weight than either the control group or the lowest treatment group ($10^{-9}\ M$). But only the oysters treated with $10^{-7}\ M$ hormone had shell weights that were significantly larger than those of the control group. Increases in total weight and wet tissue weight were not significantly different from controls; variation was high in these categories due to the large contribution of water to the weights. Tissue hydration, as measured by the dry wt/wet wt ratio, was significantly higher in the highest treatment groups when compared to the control group. There was no mortality in any of the groups.

Trout GH induced significantly higher rates of oxygen consumption in isolated gill tissue (Fig. 1). The control oxygen consumption values for both boiled rtGH and native rtGH experiments were not different and were

Table I

Effect of exogenously applied trout growth hormone on oyster growth

Treatment	Initial ht	Final ht	Total wt	Shell wt	Dry wt	Dry wt/Wet wt
Control	8.14 (.25)	11.68 (.27)	206 (11)	136 (8)	6.10 (.66)	0.219 (.019)
10^{-9} M	8.04 (.27)	11.74 (.23)	199 (9)	131 (6)	6.87 (.66)	0.262 (.023)
10^{-8} M	8.72 (.18)	12.79 (.27)*†	244 (20)	171 (11)†	9.42 (.41)*†	0.273 (.010)*
10^{-7} M	8.65 (.32)	13.00 (.36)*†	252 (15)†	189 (13)*†	9.41 (.74)*†	0.287 (.026)*

* Significantly larger than the control group (t-test; $P < 0.05$).
† Significantly larger than 10^{-9} M treatment group (t-test; $P < 0.05$).
Initial height represents mean size at the beginning of the experiment and final height, total wt, shell wt, and dry wt are mean values determined after the five-week treatment cycle was concluded. See Materials and Methods section for details. Height (ht) was measured in mm from the umbo to the ventral shell margin; weight was measured in mg. Standard errors of the mean (SEM) are in parentheses.

combined for presentation in Figure 1. Oxygen consumption in tissues treated with rtGH was significantly higher than those in tissues treated with either buffer alone or with boiled rtGH. Although the magnitude of the response was variable between individuals, the rtGH treated tissues consumed more oxygen per unit time than either control group in every experiment performed. In other experiments, insulin and bovine somatotropin stimulated oxygen consumption as well (Table II).

Discussion

We have shown that exogenously applied rtGH stimulates growth in juvenile oysters and stimulates oxygen consumption in adult gill tissues. The two highest treatment concentrations (10^{-7} and 10^{-8} M), which are considered physiological concentrations in vertebrates, elicited positive responses of similar magnitude, however, final mean sizes in the lowest treatment (10^{-9} M) group were not different from those in the control group. Although these results do not establish a growth-regulating function for rtGH, they suggest that an endocrine growth-controlling system occurring in bivalves may be related to the growth regulating system in vertebrates.

While bivalve growth has been widely studied, most experiments have focused on the relationship between growth and exogenous or environmental factors, such as water quality (seston level, chlorophyll content, temperature, and salinity). These factors have been shown to strongly influence growth rate, but recent studies have suggested that growth and the physiological functions associated with growth, such as digestion, feeding rate, and oxygen consumption, may be endogenously controlled with respect to the environment (see Bayne and Hawkins, 1990). Hawkins *et al.* (1985) concluded that feeding in mussels is time-optimized; not that nutrient gain is continuously maximized, but that feeding behavior is optimized over longer periods of time. They furthermore suggested that this feeding behavior is endogenously controlled to spare costly metabolic adjustments in response to an unstable environment, such as one typically found in the estuary.

But the growth of the juvenile oysters used in this study was much lower than that of sibling oyster groups placed in the field. Thus, the growth of the experimental oysters raised at CBI in the upwelling chambers may have been environmentally limited. Yet growth hormone treatment stimulated growth in the upwelling chambers. Therefore,
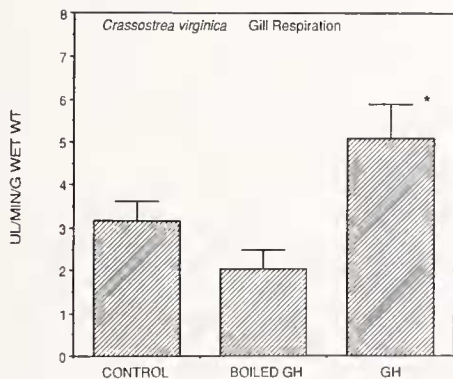

Figure 1. Oxygen consumption of isolated oyster gill tissues treated with trout growth hormone. Oxygen consumption in tissues treated with rtGH was significantly higher (paired t-test; $P < 0.05$) than in tissues treated with buffer alone or with boiled rtGH.

Table II

Mean increase in oxygen consumption in isolated oyster gill tissues produced by rtGH, insulin, or bovine somatotropin (BST) treatment

Treatment	Mean increase (μl/min/mg)	P
rtGH	3.1	.006
Insulin	2.4	.05
BST	4.2	.03

Mean increase values represent the mean increase in oxygen consumption over control gills. A paired t-test was employed to determine the significance (P) of the differences.

although sufficient nutrients must have been available for the enhanced growth to occur, the control oysters did not use them. We conclude that endogenous metabolic controls regulate growth rates with respect to, but not directly limited by, the environment, and that exogenously applied growth hormone causes a shift in that regulation. If the concentration of food in the upwellers was not limiting, the treated oysters may have fed at a greater rate to support their faster growth. On the other hand, if food levels were limiting, or nearly so, the treated oysters may have become more efficient and may have been able to assimilate and use a higher percentage of ration. These possibilities will be directly tested by performing feeding, assimilation, and utilization experiments and other scope-for-growth studies on treated and control oysters.

Our results also indicate that the effect of rtGH in oysters, like that of its evolutionary relative, prolactin, in fish (see Prosser, 1974), may be involved in osmoregulation, because it influenced tissue, and possibly cellular, hydration levels. Thus, the effect on growth could be even more indirect. Oysters grow faster at higher salinities where tissue hydration is normally slightly lower (Paynter and Mallonee, 1991; Paynter, pers. obs.). Changes in tissue hydration could affect numerous physiological functions, including feeding and digestion rates, which would ultimately lead to more rapid growth. Studies are currently underway to examine the *in vitro* effects of rtGH on various aspects of bivalve metabolism and growth.

Acknowledgments

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